Molecular Microbiology (2007) **63**(3), 711–723

doi:10.1111/j.1365-2958.2006.05552.x
First published online 4 January 2007

Fibrinogen and elastin bind to the same region within the A domain of fibronectin binding protein A, an MSCRAMM of *Staphylococcus aureus*

Fiona M. Keane,[1] Anthony Loughman,[1] Viviana Valtulina,[2] Marian Brennan,[3] Pietro Speziale[2] and Timothy J. Foster[1]*

[1]*Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Ireland.*
[2]*Department of Biochemistry, University of Pavia, Pavia, Italy.*
[3]*Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2, Ireland.*

Summary

The fibronectin binding protein, FnBPA, is a multifunctional microbial surface component recognizing adhesive matrix molecule (MSCRAMM) that promotes bacterial adherence to immobilized fibrinogen and elastin via the N-terminal A domain. The binding site for fibrinogen and elastin was localized to subdomains N2N3. A three-dimensional structural model of FnBPA was created based on the known crystal structure of the domains N2N3 of clumping factor A (ClfA). The role of individual residues in the putative ligand binding trench was examined by testing the affinity of mutants for fibrinogen and elastin. Two residues (N304 and F306) were crucial for binding both ligands and are in the equivalent positions to residues known to be important for fibrinogen binding by ClfA. A peptide comprising the C-terminus of the γ-chain of fibrinogen and a monoclonal anti-rAFnBPA antibody were potent inhibitors of the FnBPA–elastin interaction. This suggests that FnBPA binds to fibrinogen and elastin in a similar manner. Amino acid sequence divergence of 26.5% occurred between the A domains of FnBPA from strains 8325–4 and P1. Most variant residues were predicted to be located on the surface of domains N2N3 while few occurred in the putative ligand binding trench and the latching peptide explaining limited immunocross reactivity while ligand binding activity is conserved.

Accepted 30 November, 2006. *For correspondence. E-mail tfoster@tcd.ie; Tel. (+353) 1 896 2014; Fax (+353) 1 679 9294.

Introduction

Staphylococcus aureus is a commensal that colonizes the moist squamous epithelium of the human nares (Williams, 1963). It is an opportunistic pathogen that can cause superficial skin infections as well as invasive life-threatening conditions such as septic arthritis and endocarditis (Fowler *et al*., 2005).

Staphylococcus aureus expresses an array of proteins on its surface that bind to proteins in plasma and the extracellular matrix that help it to avoid phagocytosis by neutrophils and to adhere to the surface of host cells, to damaged tissue and to thrombi, thus contributing to virulence. These proteins are typified by the fibronectin binding proteins (FnBPs) which are typical microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (Signas *et al*., 1989; Jonsson *et al*., 1991). Many strains of *S. aureus* express two FnBPs called FnBPA and FnBPB that have considerable organization and sequence similarity. Most research has been performed with FnBPA from strain 8325–4. Recently the domain organization of FnBPA has been revised (Schwarz-Linek *et al*., 2003) (Fig. 1). The primary translation product has a secretory signal sequence at the N-terminus (residues 1–36) and the C-terminus contains a cell-wall anchoring domain comprising an LPXTG motif, a hydrophobic transmembrane domain and a short sequence rich in positively charged residues (residues 878–1018). The fibronectin binding region comprises 11 tandemly repeated domains spanning residues 512–877, each predicted to be capable of interacting with the type 1 modules at the N-terminus of fibronectin by a tandem β-zipper mechanism (Pilka *et al*., 2006). The binding of FnBPA to fibronectin also triggers the uptake of bacteria into non-phagocytic host cells (Peacock *et al*., 1999; Sinha *et al*., 1999) which promotes immune evasion and contributes to the persistent nature of *S. aureus* infection. Residues at the N-terminus of FnBPA (37–511) are ~20% identical to the fibrinogen binding A domain of clumping factor A (ClfA) (McDevitt *et al*., 1995) and are predicted to fold into three subdomains N1, N2 and N3 similar to ClfA (Deivanayagam *et al*., 2002). The A domains of FnBPA and

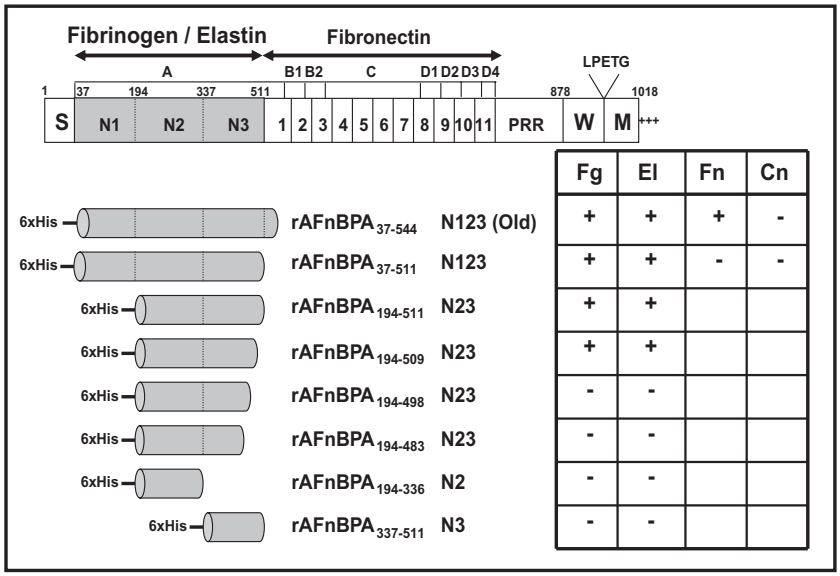

Fig. 1. Structural organization of the fibronectin-binding protein, FnBPA from *S. aureus* strain 8325–4 and recombinant truncated derivatives. A secretory signal sequence (S), region A (divided into subdomains N1, N2 and N3), regions B, C and D (now tandem repeats 1–11), cell wall-spanning domain (W) and membrane-spanning domain (M) are indicated. The LPXTG motif, involved in anchoring the protein to the cell wall peptidoglycan, is indicated. The recombinant rAFnBPA proteins with N-terminal 6xHis tags are also shown along with a table summarizing the ability of each truncate to bind to relevant ligands, fibrinogen (Fg), elastin (El), fibronectin (Fn) and collagen (Cn).

ClfA bind to fibrinogen at the C-terminus of the γ-chain (Wann *et al*., 2000). Unlike ClfA, the A domain of FnBPA also binds to elastin (Roche *et al*., 2004). One of the objectives of this study was to determine if elastin and fibrinogen bound to similar or distinct regions of FnBPA.

The A domain of the MSCRAMM SdrG/Fbe protein from *Staphylococcus epidermidis* has structural similarity to ClfA (and FnBPA). The X-ray crystal structures of apo forms of ClfA and SdrG A domains have been solved (Deivanayagam *et al*., 2002; Ponnuraj *et al*., 2003). The structure of SdrG in complex with the fibrinogen β-chain peptide has also been solved. A molecular model for SdrG binding to the fibrinogen β-chain has been proposed and is called 'dock-lock-latch' (Ponnuraj *et al*., 2003). The peptide binds to a groove located between domains N2 and N3 in the apo form. C-terminal residues of domain N3 undergo a conformational change to bind adjacent to a β-strand in domain N2 forming an extra β-strand termed the latching peptide. This traps the fibrinogen peptide in its grove between N2 and N3 and locks it in place. The structural similarity between ClfA and SdrG suggests that a similar mechanism of ligand binding occurs. This is supported by *in silico* docking of the γ-chain peptide into ClfA and by amino acid substitutions of residues located in the putative ligand binding trench which caused defects in fibrinogen binding (Deivanayagam *et al*., 2002). Most notably substitution of residues Pro 336 and Tyr 338 on cell surface expressed ClfA, caused complete loss of ligand binding (Loughman *et al*., 2005).

Elastin is a polymeric protein that provides resilience and elasticity to skin, blood vessels, lungs, ligaments and tendons. The elastin precursor, tropoelastin, which is composed of alternating hydrophobic and hydrophilic domains, is secreted during foetal development by smooth muscle cells, endothelial cells and fibroblasts (Rosenbloom, 1982). In a process called coacervation, tropoelastin monomers become aligned and lysine residues located in hydrophilic domains are cross-linked by lysyl oxidase to form insoluble elastin (Cox *et al*., 1974). Commercial elastin peptides are produced by boiling elastin-rich tissue in oxalic acid which cleaves at hydrophilic sites. The resulting soluble elastin peptides are heterogeneous in nature. They are routinely used in binding experiments because they retain unique cross-links and bind the same cellular receptors and host proteins as intact polymeric elastin.

The initial analysis of the binding of *S. aureus* to elastin identified the elastin binding protein EbpS, a membrane-associated protein which is capable of binding to elastin peptides in solution (Park *et al*., 1996; Downer *et al*., 2001) but which is not an MSCRAMM because it does not support adherence of bacterial cells to elastin peptides immobilized on a solid surface. However, many clinical strains of *S. aureus* adhere strongly to immobilized elastin. This was shown to be mediated by the fibronectin binding proteins and subsequent molecular analysis showed that elastin bound to the A domain of FnBPA (Roche *et al*., 2004).

The aim of this study was to investigate the binding site(s) within the A domain of FnBPA for fibrinogen and elastin and to determine if the ligands bound to the same or different regions. This was achieved by expressing truncated derivatives and substitution mutants of the A domain of the 8325–4 FnBPA protein and comparing affinities for the two ligands in solid phase assays and by analysis of the A domain of FnBPA from strain P1 which differs in amino acid sequence from 8325–4.

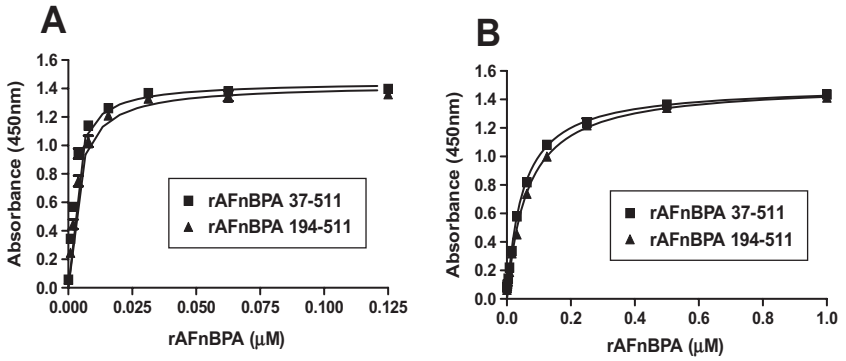

Fig. 2. Binding of recombinant FnBPA truncates, rAFnBPA$_{37–511}$ and rAFnBPA$_{194–511}$ to immobilized human fibrinogen (A) and elastin (B). Microtitre wells were coated with 10 μg ml^{-1} human fibrinogen or elastin and increasing concentrations of rAFnBPA truncates were added for 1 h. Bound protein was detected with polyclonal anti-rAFnBPA antibodies, HRP-conjugated goat anti-rabbit antibodies followed by TMB substrate. Values represent the mean of triplicate wells.

Results

Binding of the recombinant A domain of FnBPA to elastin and fibrinogen

It was reported previously that the A domain of FnBPA of *S. aureus* strain 8325–4 comprising residues 37–544 bound to immobilized elastin and to fibrinogen (Wann *et al*., 2000; Roche *et al*., 2004). The co-ordinates of FnBPA were redefined (Schwarz-Linek *et al*., 2003) and it was noted that rAFnBPA$_{37–544}$ likely contained a fibronectin-binding domain in C-terminal residues 512–544. Its ability to bind fibronectin was confirmed by us (data not shown). New constructs lacking the fibronectin-binding region and comprising only domains N1, N2 and N3 (rAFnBPA$_{37–511}$) and a shorter construct lacking domain N1 (rAFnBPA$_{194–511}$) were expressed with 6xHis affinity tags at their N-termini. The two proteins bound to immobilized fibrinogen with similar half-maximum binding, in the low nanomolar range (rAFnBPA$_{37–511}$ half maximum binding = 4.7 ± 1.8 nM, rAFnBPA$_{194–511}$ half maximum binding = 4.02 ± 0.23 nM) and to elastin with half maximum binding that was about 10- to 12-fold higher (rAFnBPA$_{37–511}$ half maximum binding = 45.6 ± 10.3 nM, rAFnBPA$_{194–511}$ half maximum binding = 51.77 ± 12.2 nM) (Fig. 2). This indicates that neither the N-terminal N1 domain nor residues 512–544 are required for binding to either ligand.

Modelling the three-dimensional (3D) structure of FnBPA 194–511

The A domain of FnBPA has ~20% amino acid identity to the A domain of ClfA and binds to the same residues in the C-terminus of the γ-chain of fibrinogen. A model of the 3D structure of domains N2 and N3 of FnBPA was constructed based on the known 3D structure of the apo form of ClfA. In addition, a peptide comprising the C-terminal eight residues of the γ-chain of fibrinogen (G_{404}AKQAGDV_{411}) was docked *in silico* into the putative binding trench between domains N2 and N3 in order to help predict residues that might be involved in ligand binding (Fig. 3). The resulting 3D molecular model would inform our studies on residues involved in ligand binding and in antigenicity.

Mutants of rFnBPA lacking C-terminal residues

One of the objectives of this project was to construct mutants of FnBPA that would help determine if elastin and fibrinogen bind to similar or distinct regions of the A domain. In order to investigate the role of the C-terminal residues of the shortened rAFnBPA N2N3 construct (rAFnBPA$_{194–511}$) in ligand binding, several C-terminally truncated derivatives were constructed and purified. rAFnBPA$_{194–509}$ lacks two C-terminal residues predicted to form the end of the latching β strand (Fig. 3). This protein bound to fibrinogen with a statistically significant difference in half maximum binding compared with that of rFnBPA$_{194–511}$ (half maximum binding of rAFnBPA$_{194–511}$ = 4.02 ± 0.23 nM, half maximum binding of rAFnBPA$_{194–509}$ = 20.94 ± 7.5 nM: *P*-value = 0.0175) whereas there was no significant difference in the half maximum binding for elastin (Fig. 4). In contrast, a further deletion, rFnBPA$_{194–498}$, did not bind detectably to either ligand (Fig. 4). These results are consistent with FnBPA binding both ligands by a 'dock lock latch' mechanism because rAFnBPA$_{194–498}$ lacks residues that would comprise the latching peptide and the hinge region covering the ligand-occupied trench. A third construct, rAFnBPA$_{194–483}$, as well as individual subdomains N2 (rAFnBPA$_{194–336}$) and N3 (rAFnBPA$_{337–511}$), were also unable to bind fibrinogen or elastin (data not shown).

Amino acid substitution mutants of FnBPA

In order to investigate in more detail if FnBPA binds to fibrinogen and elastin at the same or different sites, a series of amino acid substitutions were introduced in rAFnBPA$_{37–511}$ in residues predicted to be located in the trench between domains N2 and N3 that might be involved in ligand binding. Residues were targeted for alteration because their side chains were predicted by

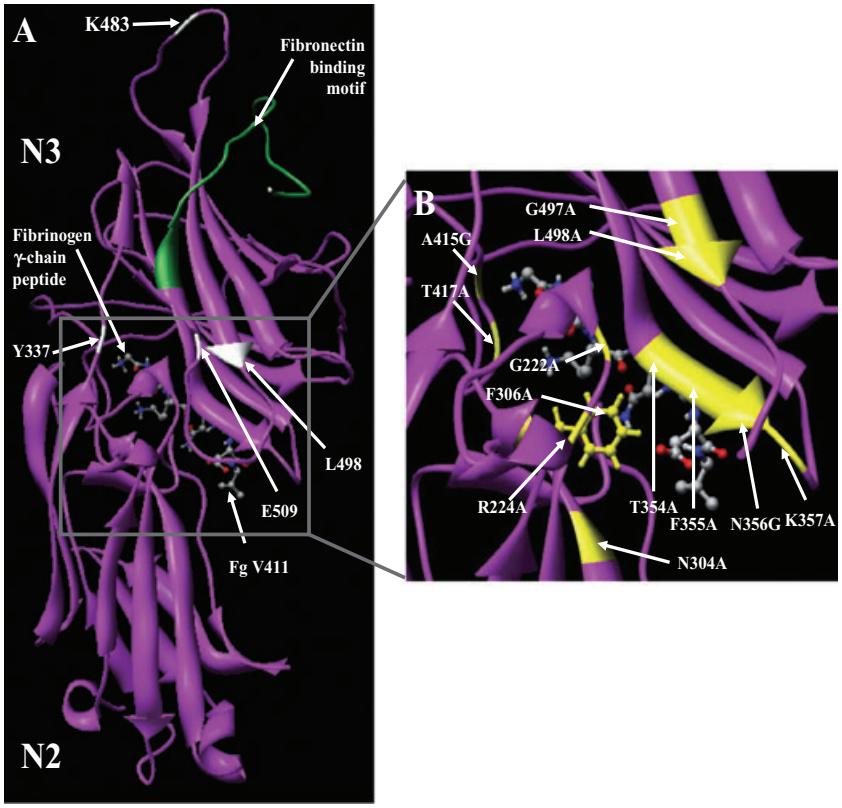

Fig. 3. Docking of fibrinogen γ-chain peptide (G_{404}AKQAGDV$_{411}$) into the predicted 3D structure of rAFnBPA N23, based on homology modelling with equivalent domains of ClfA.
A. Entire structure. Green shows the fibronectin binding domain at the end of N3, while successive C-terminal deletion constructs are indicated in white (194–509, 194–498 and 194–483). The boundary between N2 and N3 (Y337) is also indicated in white.
B. Closer view of the ligand binding trench. Residues targeted for site-directed mutagenesis are shown in yellow with the side chain of the F306 residue shown in ball and stick format.

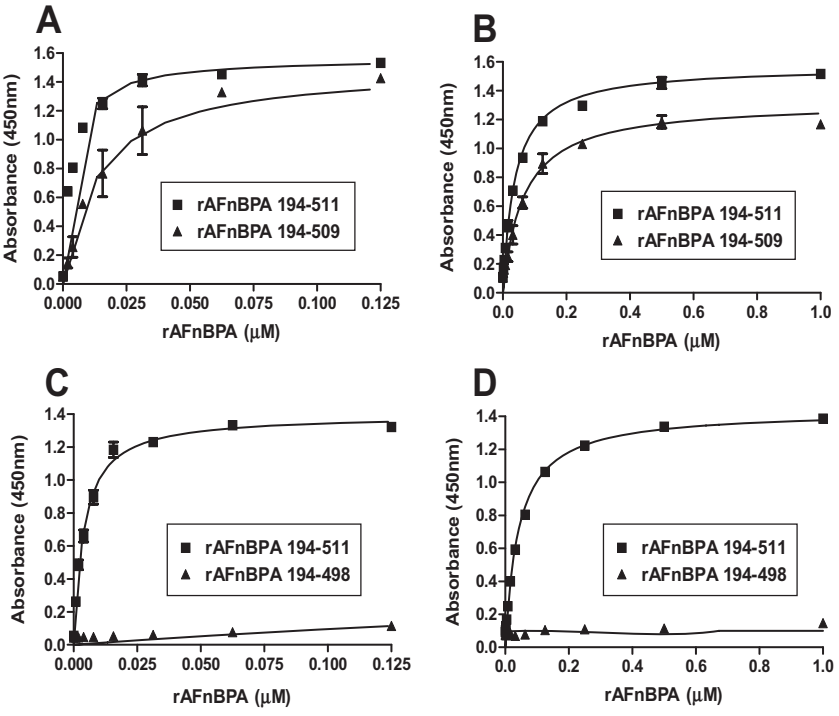

Fig. 4. Binding of recombinant FnBPA truncates, rAFnBPA$_{194–511}$ and rAFnBPA$_{194–509}$ (A and B) and rAFnBPA$_{194–511}$ and rAFnBPA$_{194–498}$ (C and D) to immobilized human fibrinogen (A and C) and elastin (B and D). Microtitre wells were coated with 10 μg ml^{-1} human fibrinogen or elastin and increasing concentrations of rAFnBPA truncates were added for 1 h. Bound protein was detected with polyclonal anti-rAFnBPA antibodies, HRP-conjugated goat anti-rabbit antibodies followed by TMB substrate. Values represent the mean of triplicate wells.

Table 1. The relative affinities of rAFnBPA$_{37–511}$ wild type and 11 amino acid-substituted proteins were assessed by ELISA-type binding assays to immobilized fibrinogen and elastin.

	Fibrinogen		Elastin	
Protein	Half maximum binding ± SD (nM)	*P*-value	Half maximum binding ± SD (nM)	*P*-value
rAFnBPA$_{37–511}$ wild type	4.7 ± 1.8	–	45.6 ± 10.3	–
rAFnBPA$_{37–511}$ G222A	6.7 ± 1.5	0.2134	288.4 ± 145.6	0.05
rAFnBPA$_{37–511}$ R224A	120.9 ± 28.8	0.0022	520.6 ± 169	0.0083
rAFnBPA$_{37–511}$ N304A	1032.2 ± 112	0.0001	1227.3 ± 106	0.0001
rAFnBPA$_{37–511}$ F306A	ND	ND	ND	ND
rAFnBPA$_{37–511}$ F355A	412.5 ± 205	0.0262	236.6 ± 182.5	0.1446
rAFnBPA$_{37–511}$ K357A	31.5 ± 14.2	0.0316	68.1 ± 27.1	0.25
rAFnBPA$_{37–511}$ G497A	82.2 ± 14.9	0.0009	665.7 ± 156.4	0.0024
rAFnBPA$_{37–511}$ L498A	682.1 ± 96	0.0003	959.0 ± 368.5	0.0127
rAFnBPA$_{37–511}$ N304A/F306A	ND	ND	ND	ND
rAFnBPA$_{37–511}$ T354A/N356G	23.9 ± 14.9	0.091	370.1 ± 97.6	0.0046
rAFnBPA$_{37–511}$ A415G/T417A	697.7 ± 266.7	0.0108	630.2 ± 227.5	0.0113

Half maximum binding values given are approximated by the GraphPad Prism software package and are averaged over at least three experiments. The *P*-value given represents the statistical significant difference between the binding for each variant compared with that of rAFnBPA$_{37–511}$ wild type for the binding to each ligand.
ND, no detectable binding observed.

the Chimera programme to be very close to the docked γ-chain of fibrinogen (T354, N356, A415, T417) and because residues in the equivalent position in the ligand binding trench of ClfA were previously shown to be important (G222, R224, N304, F306, F355 and K357) (Deivanayagam *et al*., 2002). Two residues (G497 and L498) were also included as they are in the equivalent position to those identified in a previous empirical study of ClfA ligand binding (E526 and V527) (Hartford *et al*., 2001). The far-UV circular dichromism (CD) spectra of three mutant proteins were similar to that of wild-type rAFnBPA$_{37–511}$ protein (data not shown), suggesting that the substitutions did not dramatically alter the conformation of the protein. This was supported by each purified variant protein reacting with similar affinity to five monoclonal anti-rAFnBPA antibodies (data not shown).

In each case binding of the purified recombinant protein to elastin and fibrinogen was measured in solid phase assays and the apparent half maximum binding values recorded (Table 1). The effects of alterations to the affinity of recombinant FnBPA for fibrinogen and elastin fell into four categories: (i) no significant reduction in fibrinogen or elastin binding (G222A), (ii) small reductions in fibrinogen binding and greater, more significant reductions in elastin binding (T354A/N356G), (iii) significant reductions in affinity for fibrinogen with no reduction in elastin binding (F355A, K357A) and (iv) significant reductions in binding to both ligands (R224A, N304A, F306A, N304A/F306A, A415G/T417A, G497A and L498A). It is noteworthy that the residues that line the base of the trench between N2 and N3 all fall into the last class which strongly supports the notion that both fibrinogen and elastin bind to the same region. Alterations in residues comprising the β-strand that lines the upper part of the trench have less severe effects (T354, F355, N356, K357) although T354A/N356G does significantly affect elastin binding. F355 and K357 are not predicted to be in close contact with fibrinogen so the reductions in binding might be indirect due to a localized alteration in conformation. Residue A415 is predicted to be in contact with G404 of the fibrinogen γ chain peptide, while T417 is located very near to the end of the side chain of K406 of fibrinogen. The side chain amino group of R224 is close to the carboxyl group at the end of D410 of fibrinogen suggesting a possible ionic interaction. It is particularly notable that residues N304 and F306 that are in equivalent positions to two crucial residues in ClfA (P336, Y338) show the greatest defects in ligand-binding when converted to alanine (Fig. 5). The side chains of both N304 and F306 appear to interact with the side chain of D410 in the fibrinogen γ chain peptide. Substitutions G497A and L498A exhibited defects in fibrinogen binding and elastin binding. Residues G497 and L498 are not predicted to contact the fibrinogen peptide and are more likely to be involved in the latching and locking mechanism rather than peptide binding. These results are consistent with elastin and fibrinogen binding to the same region of FnBPA with some differences in amino acids in FnBPA that are involved in contacts with the ligands.

The N304A/F306A double substitution was introduced into the FnBPA protein expressed by pNZ8037 in *L. lactis*. Whole cell immunoblotting experiments indicated that both proteins were expressed at similar levels (data not shown). *L. lactis* expressing the mutant protein did not adhere detectably to immobilized fibrinogen or elastin but retained ability to adhere to fibronectin (Fig. 6) indicating functional integrity of the surface-expressed FnBPA while

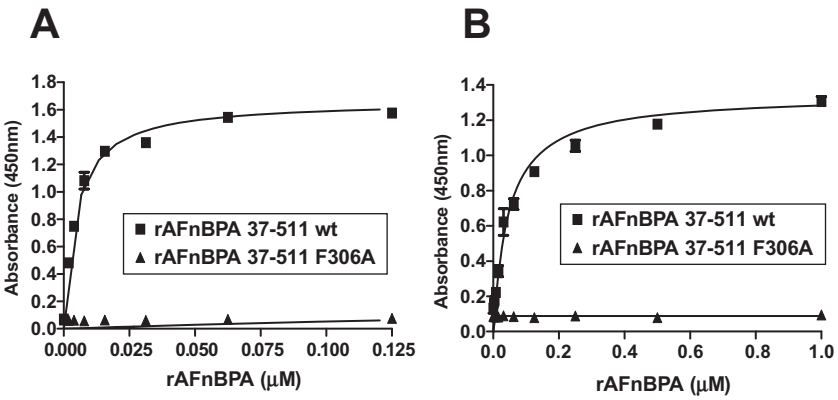

Fig. 5. Binding of recombinant FnBPA site-directed mutants, rAFnBPA$_{37–511}$ wild type and rAFnBPA$_{37–511}$ F306A to immobilized human fibrinogen (A) and elastin (B). Microtitre wells were coated with 10 μg ml^{-1} human fibrinogen or elastin and increasing concentrations of rAFnBPA proteins were added for 1 h. Bound protein was detected with polyclonal anti-rAFnBPA antibodies, HRP-conjugated goat anti-rabbit antibodies followed by TMB substrate. Values represent the mean of triplicate wells.

confirming results obtained with recombinant rAFnBPA$_{37–511}$ N304A or F306A protein.

Inhibition of adherence of L. lactis *(FnBPA) to immobilized fibrinogen and elastin*

Lactococcus lactis expressing the full length FnBPA protein on the bacterial cell surface was used to compare the effect of known inhibitors of fibrinogen binding on bacterial adherence to immobilized elastin. A 17 amino acid peptide representing the C-terminus of the γ-chain was previously shown to inhibit recombinant rAFnBPA binding to fibrinogen (Wann *et al*., 2000). Here dose-dependent and saturable inhibition of adherence of *L. lactis* FnBPA to both fibrinogen and elastin was observed. Maximum inhibition of adherence to fibrinogen (~70%) was achieved only at the highest concentration of peptide (400 μM) whereas ~85% inhibition of adherence to elastin was achieved with 100 μM peptide. In both cases the scrambled peptide had no effect (Fig. 7). The more potent inhibition of binding to elastin by the fibrinogen peptide possibly reflects the higher affinity of FnBPA for fibrinogen compared with elastin. The inhibition of elastin binding with the fibrinogen γ chain peptide reflects the inability of both ligands to bind simultaneously to rAFnBPA.

Monoclonal antibody 7C5 was previously shown to inhibit FnBPA-expressing bacteria from adherence to fibrinogen (Fitzgerald *et al*., 2006). Here the ability of the antibody to inhibit elastin binding was tested. The antibody inhibited adherence of *L. lactis* FnBPA to immobilized fibrinogen to a maximum of 60%. 7C5 also strongly inhibited adherence of *L. lactis* to immobilized elastin with

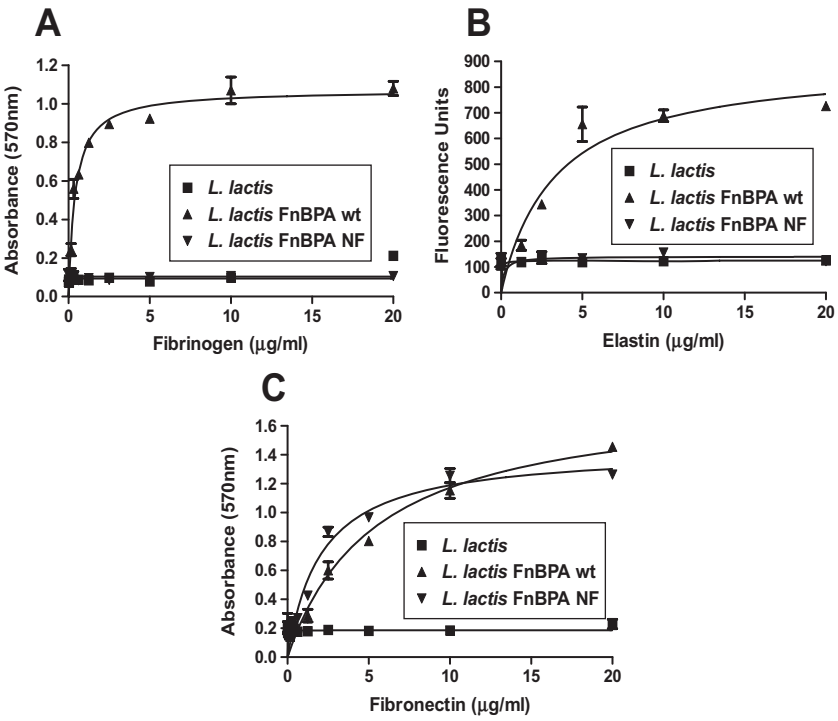

Fig. 6. Adherence of *L. lactis* strains expressing full length FnBPA wild type or FnBPA with N304A/F306A substitutions to immobilized fibrinogen (A), elastin (B) and fibronectin (C). Washed cells (OD = 1.0) were added to ligand coated wells for 1.5 h. Adherent bacteria to fibrinogen and fibronectin were stained with crystal violet and the absorbance at 570 nm measured in an ELISA plate reader. Elastin adherence was measured by preincubating cells with SYTO-13 nucleic acid stain followed by reading in a fluorescence spectrophotometer.

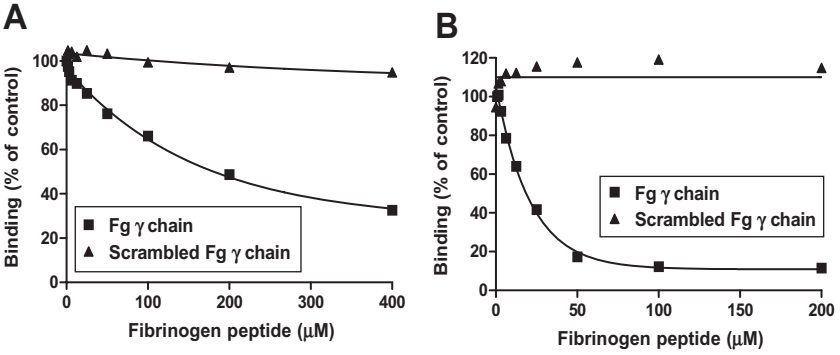

Fig. 7. Inhibition of *L. lactis* expressing FnBPA binding to immobilized fibrinogen (A) and elastin (B) by preincubation with fibrinogen γ chain peptide. Microtitre wells were coated with 10 μg ml^{-1} of human fibrinogen or elastin. Cells preincubated with final concentrations of 400 μM to 0 μM of fibrinogen γ chain or a scrambled version of this peptide were added to each well. Adherent bacteria were stained with crystal violet or SYTO-13. Values are expressed as a percentage of control wells lacking inhibitor.

~80% inhibition being achieved at 50 μg ml^{-1} of antibody. An isotype-matched control monoclonal antibody, 1F9 was known not to inhibit fibrinogen binding. This antibody failed to inhibit elastin binding (Fig. 8).

These experiments are consistent with the interpretation that fibrinogen and elastin bind to FnBPA at sites that overlap.

Antigenic differences in the A domain of FnBPA from S. aureus *strain 8325–4 and P1*

Previously we showed that the FnBPs expressed by *S. aureus* strain P1 were necessary to promote bacterial adherence to immobilized elastin (Roche *et al*., 2004). However, immunoblotting experiments with proteins released from whole cells suggested that the FnBPA protein of P1 reacted weakly with polyclonal antibodies raised against the A domain of FnBPA from strain 8325–4 (data not shown). A report that *fnb* genes from diverse strains of *S. aureus* have undergone greater sequence divergence than other surface protein genes such as *clf*B and *clfA* (Kuhn *et al*., 2006) prompted us to examine more closely the A domain of FnBPA from strain P1. DNA coding for the entire A domain was amplified by polymerase chain reaction (PCR), sequenced and the amino acid sequence deduced and compared with that of strain 8325–4. The overall amino acid sequence identity was ~73.5% but closer examination indicated that the greatest divergence occurred in domains N2 and N3. The N1 domains are 91.4% identical; the N2 domains are 78.3% identical while the N3 domains are only 59.2% identical. The variant residues were studied in the context of the 3D model of domains N2 and N3 of 8325–4 and most were predicted to be exposed on the surface of the protein. Some changes occurred within the putative ligand-binding trench, the putative latching peptide and the slot in N2 into which the latching peptide would insert during 'dock-lock-latch' (Fig. 9). Residues targeted by mutagenesis were mostly conserved between the two proteins with the exception of N356 and T417 of 8325–4 being present as D356 and I417, respectively, in P1. A recombinant form of the N2N3 domains of the P1 protein was expressed and its affinity for fibrinogen and elastin was compared with the equivalent protein from 8325–4. The P1 protein bound to both ligands (data not shown). It had the same affinity for fibrinogen as the 8325–4 protein but interestingly it had a significantly higher affinity for elastin (half maximum binding $rAFnBPA_{194–511}$ 8325–4 for elastin = 51.77 ± 12.2 nM; half maximum binding $rAFnBPA_{194–511}$ P1 for elastin = 4.12 ± 3.8 nM, *P*-value = 0.003). The variant residues observed for P1 therefore do not adversely affect ligand binding with those changes situated around the putative ligand binding trench or latching peptide possibly involved in the increased adherence to elastin.

The ability of FnBPA from P1 to bind to polyclonal and monoclonal antibodies raised against the 8325–4 protein

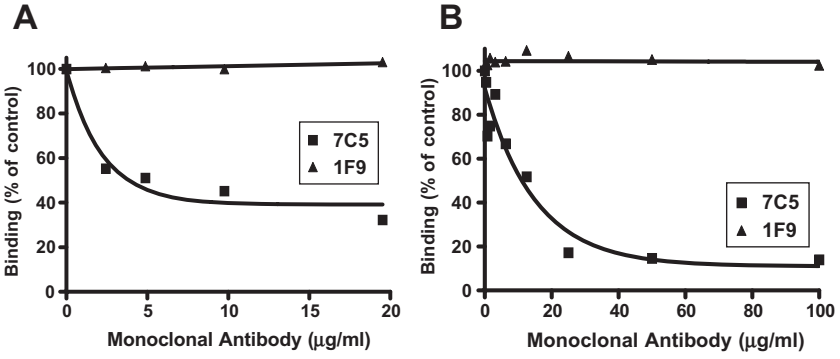

Fig. 8. Inhibiton of *L. lactis* expressing FnBPA binding to immobilized fibrinogen (A) and elastin (B) by preincubation with monoclonal antibody 7C5. Microtitre wells were coated with 10 μg ml^{-1} of human fibrinogen or elastin. Cells preincubated with final concentrations of 100 μg ml^{-1} to 0 μg ml^{-1} of monoclonal antibody 7C5 or a control monoclonal antibody (1F9) were added to each well. Adherent bacteria were stained with crystal violet or SYTO-13. Values are expressed as a percentage of control lacking inhibitor.

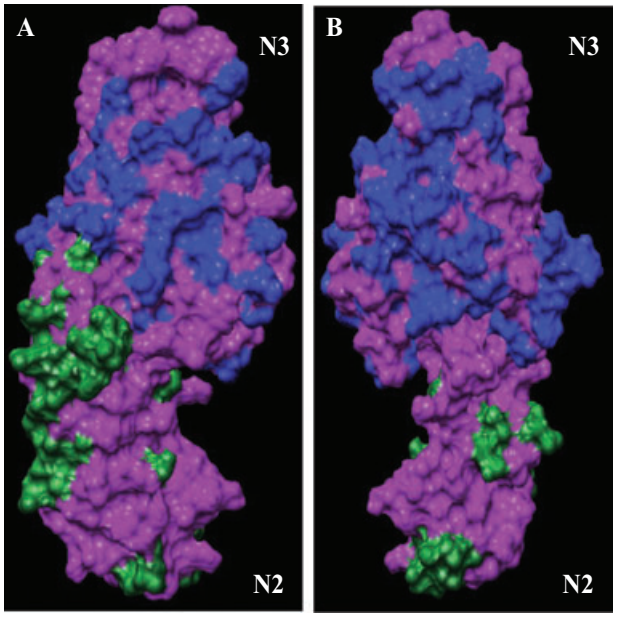

Fig. 9. Space filled model of the predicted 3D structure of the N23 domains of region A of FnBPA from *S. aureus* strain 8325–4 with those residues that differ in FnBPA of strain P1 highlighted in green (N2) and blue (N3).
A. Space filled model in the orientation as per ribbon diagram in Fig. 3.
B. Space filled model of same protein turned 180°.

was tested. Different amounts of both proteins ranging from 0.125 to 1.0 μg were subjected to SDS-PAGE and Western immunoblotting (Fig. 10). Densitometry indicated that the polyclonal antibodies had a four to 12-fold lower affinity for the P1 protein compared with that for 8325–4. Four monoclonal antibodies failed to react detectably with the P1 protein including the function blocking monoclonal antibody, 7C5.

These results show that the ability of FnBPA from strain P1 to bind fibrinogen and elastin has been conserved while substantial changes have occurred to residues on the surface of the protein involved in antibody recognition but not involved in ligand binding. Despite the changes observed in FnBPA from strain P1, this protein can still bind fibrinogen and elastin which is consistent with both ligands binding to the same region and possibly by the same mechanism.

Discussion

The colonization of host tissue by *S. aureus* is an important factor in disease pathogenesis. *S. aureus* expresses on its cell surface a number of MSCRAMMs that promote the binding of the organism to components of the host extracellular matrix and play an important role in bacterial virulence. *S. aureus* can infect elastin-rich tissues such as lung, skin, joints and heart valves. It is likely to encounter elastin on its journey through blood vessel walls, traversing the extracellular space beneath the basement membrane of endothelial cells in tissue such as lung and around the synovial lining of various joints. This is in contrast to fibrinogen-rich sites such as the bloodstream and the conditioned surface of biomaterials. Here the

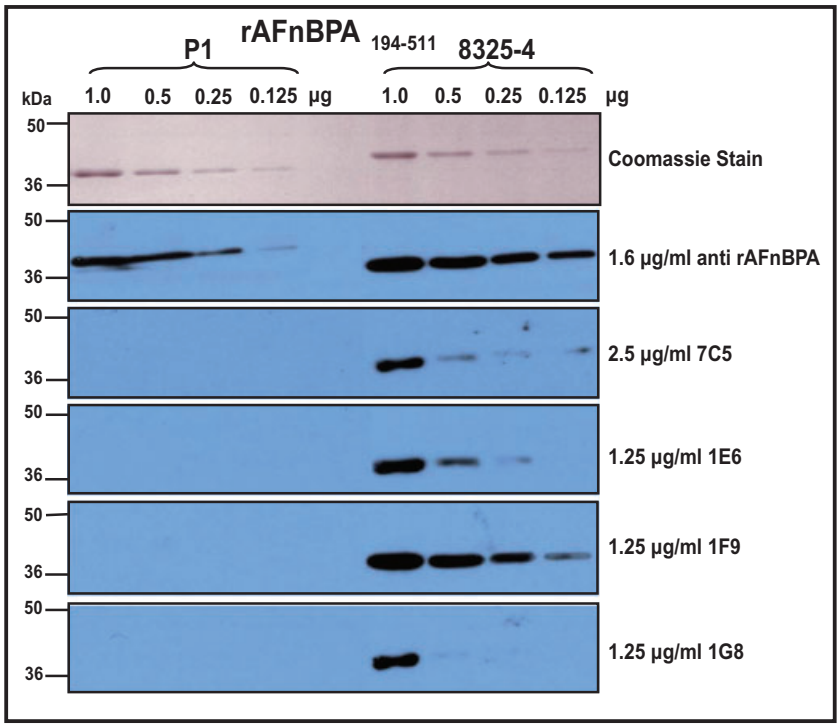

Fig. 10. Coomassie and Western blot analysis of rAFnBPA$_{194–511}$ from *S. aureus* strains 8325–4 and P1. Doubling dilutions of protein (1.0 μg – 0.125 μg) were subjected to SDS-PAGE and stained with Coomassie Blue or transferred to PVDF membrane and probed with polyclonal anti-rAFnBPA antibodies (1.6 μg ml^{-1}) or monoclonal antibodies 7C5 (2.5 μg ml^{-1}), 1E6, 1F9 and 1G8 (1.25 μg ml^{-1}).

interaction of *S. aureus* with elastin was examined. The ability of *S. aureus* FnBPA A domain to bind to immobilized elastin peptides was reported previously (Roche *et al*., 2004). The fibronectin-binding region at the C-terminus of FnBPA has been studied in detail and 11 tandemly repeated binding domains were mapped (Fig. 1) (Schwarz-Linek *et al*., 2003). It was noted that a single fibronectin binding domain was located at the C-terminus of the recombinant A domain protein used in previous fibrinogen and elastin binding assays (Roche *et al*., 2004). This protein was shown to bind immobilized fibronectin which indicates that the presence of one fibronectin binding motif is sufficient to interact with this ligand. The fibrinogen/elastin and fibronectin binding domains of FnBPA are very closely located at this junction, which could have implications in competitive ligand binding *in vivo*. The revised A domain of FnBPA spanning residues 37–511 was expressed. This protein and an N23 truncate (residues 194–511) were shown to be capable of binding fibrinogen and elastin but not fibronectin. This indicates that, similar to ClfA and ClfB, the N1 subdomain is not required for ligand binding. The binding site for both fibrinogen and elastin was thus localized to residues 194–511 of FnBPA.

A 3D molecular model of the N2 and N3 domains of FnBPA based on the known structure of ClfA facilitated analysis of the binding sites for elastin and fibrinogen. Initially residues were removed from the C-terminus of the revised N2N3 construct, rAFnBPA$_{194–511}$. Removal of two residues reduced the affinity for fibrinogen but not for elastin while a truncate rAFnBPA$_{194–498}$ lacking an additional 10 residues did not bind detectably to either ligand. This is consistent with both ligands binding to the same region of FnBPA possibly involving the dock-lock-latch mechanism.

In order to define further the ligand binding sites in FnBPA, amino acid substitutions were created in the putative ligand binding trench located between N2 and N3. The reduction in ligand binding seen with various substitutions can be correlated with their position around the hydrophobic trench and the predicted interactions of their side chains with residues of the fibrinogen peptide. The glycine residue at position 222 does not have a role to play in ligand binding whereas the substitution of another glycine, G497 does have an effect on ligand binding. This can be explained by G222 being located within the hydrophobic trench but it is some distance from the fibrinogen ligand. In contrast, G497 is located at the junction of the penultimate β strand of N3 and the loop segment that most likely acts as the hinge that traverses the hydrophobic trench when the ligand is bound. This glycine residue could have a role in bending the hinge region over the fibrinogen ligand. The importance of the hydrophobic residue L498 might be its repositioning after the conformational change during latching and locking where it might stabilize the binding as occurs with Ile581 that is in a similar position in the ligand bound complex of SdrG (Ponnuraj *et al*., 2003). Residues N304 and F306 were shown to be particularly important for binding both ligands and are predicted to be in close proximity to the side chain of D410 of fibrinogen. They are located in equivalent positions to the crucial residues P336 and Y338 of ClfA (Deivanayagam *et al*., 2002; Loughman *et al*., 2005). This was confirmed by introducing N304A and F306A substitutions into FnBPA expressed on the surface of *L. lactis*. The importance of the ligand binding trench was reinforced by the reduced affinity exhibited by substitutions of several other residues. These data strongly suggest that both elastin and fibrinogen bind to the same region of FnBPA. This was supported by the strong inhibition of adherence of *L. lactis* (FnBPA) to elastin by the C-terminal fibrinogen γ chain peptide.

The ability of the γ-chain peptide of fibrinogen to block binding to elastin suggests that the two ligands compete for the same binding site. However, the weaker binding of FnBPA for elastin compared with fibrinogen should not preclude a biologically significant interaction occurring in niches where the latter ligand is abundant and fibrinogen is absent.

Elastin is a highly cross-linked polymer of tropoelastin. There is no obvious amino acid sequence similarity between tropoelastin and the γ chain of fibrinogen comprising the known FnBPA binding site. Without a crystallized complex of the ligands bound to FnBPA it is impossible to determine how two different proteins can binds to the same site. However, the ability of a *S. aureus* surface protein to bind unrelated ligands is not without precedent because we have recently shown that the same face of the staphylococcal Protein A domain D binds to the Fc region of IgG, to the A1 and D'D3 domains of von Willebrand factor (O'Seaghdha *et al*., 2006) and to tumour necrosis factor receptor-1 (Gomez *et al*., 2006).

The elastin substrate used *in vitro* binding assays is a partially degraded form of human aortic elastin that retains cross-links but which has free N- and C-terminal peptides caused by chemical cleavage in hydrophilic domains. It is likely that these bind to the ligand binding trench in FnBPA. During infection it is possible that *S. aureus* encounters similar peptide fragments created by cleavage of host tissue by bacterial or host elastases. It has been shown previously that the elastin preparations used in binding assays are not contaminated with fibrinogen or fibronectin (Roche *et al*., 2004), indicating the specificity of the FnBP–elastin interaction.

It has been reported that the *fnbA* and *fnbB* genes from 50 different strains representing the major MRSA clones found in Europe have undergone greater sequence divergence than genes encoding other surface proteins such

as *clfA* and *clfB* (Kuhn *et al*., 2006). The amino acid sequence of the A domain of FnBPA of strain P1 was shown here to be 73.5% identical to that of 8325–4. Interestingly the differences were not spread evenly. Domain N1 was more highly conserved while domain N3 was the most divergent. Using the 3D model of the 8325–4 FnBPA A domain it is evident that the majority of variant residues are located on the surface of the protein. Residues identified by mutagenesis to be important in ligand binding were mostly conserved. The consequences of this sequence divergence were reduced ability of antibodies raised against the 8325–4 protein to bind to the P1 protein while retaining the ability to bind to both fibrinogen and elastin. This suggests that the ability of FnBPA to bind these ligands during infection is important and further supports our contention that binding of fibrinogen and elastin occurs at the same site. We are currently extending this investigation to include FnBPs from a variety of clonal types in order to determine the extent of the sequence divergence and antigenic variation, and their relevance to evasion of immune responses during infection.

Experimental procedures

Bacterial strains and growth conditions

Cloning was routinely performed in *Escherichia coli* strain XL-1 Blue (Stratagene). *E. coli* strains were transformed by the calcium chloride method (Sambrook. J., 1989). *E. coli* strain TOPP 3 (Qiagen) was used for the expression of recombinant truncates of FnBPA. *S. aureus* strain P1 (Sherertz *et al*., 1993) is a derivative of ATCC 25923. *Lactococcus lactis* strain NZ9800 (De Ruyter *et al*., 1996) and the nisin inducible vector expressing the *fnbA* gene of *S. aureus* 8325–4, pNZ8037*fnbA* (Fitzgerald *et al*., 2006) were used to study the binding ability of FnBPA in isolation from other ligand binding *S. aureus* surface proteins. *L. lactis* was routinely grown on M17 agar or statically in M17 broth incorporating 0.5% glucose and induced with nisin (Sigma) at 30°C. Ampicillin (100 μg ml^{-1}) and chloramphenicol (10 μg ml^{-1}) were incorporated into the growth medium where appropriate.

Expression and purification of recombinant proteins

Regions of the 8325–4 *fnbA* gene encoding amino acids 37–544, 37–511, 194–511, 194–509, 194–498, 194–483, 194–336, 337–511 were PCR-amplified from plasmid pQE30-FnBPA$_{37–544}$ (Roche *et al*., 2004) using primers (sequences available on request) incorporating BamHI or SalI restriction sites. The PCR products were cloned into the N-terminal six-histidine tag expression vector pQE30 (Qiagen). Recombinant proteins were expressed and purified by Ni^{2+} chelate chromatography followed by Q-Sepharose chromatography as described previously (O'Connell *et al*., 1998). The A domain-encoding region of the P1 *fnbA* gene was amplified using 8325–4-based primer pairs homologous to the more conserved flanking sequences present in the signal sequence and B repeat encoding regions. The resulting product was ligated into pBluescript (Stratagene) and sequenced, allowing more specific primers to be designed to clone the precise N23 subdomain-encoding sequence into pQE30. The resulting recombinant protein was purified as above.

Antibodies

Antibodies reactive against the A domain of FnBPA were obtained by immunizing specific pathogen-free rabbits with rAFnBPA$_{37–544}$ (Roche *et al*., 2004). Immunoglobulin was purified by the method of Owen (Owen, 1985). Monoclonal antibodies 7C5 (Fitzgerald *et al*., 2006), 1E6, 1F9 and 1G8 were obtained by immunizing mice with rAFnBPA$_{37–544}$. Goat anti-rabbit antibodies and rabbit anti-mouse antibodies, both conjugated to horseradish peroxidase (HRP) were purchased from Dako, Denmark.

Direct binding of recombinant FnBPA proteins to immobilized elastin and fibrinogen

Human aortic elastin (Elastin Products Company; 10 μg ml^{-1}) and human fibrinogen (Enzyme Research Laboratories; 10 μg ml^{-1}) were coated onto microtitre wells for 18 h under UV light and at 4°C respectively. Plates were washed three times with phosphate-buffered saline (PBS) and blocked with 5% skimmed milk in Tris saline (TS) buffer (10 mM Tris-HCl pH 7.4, 150 mM NaCl) containing Tween 20 (0.05% v/v) for 2 h at 37°C. Following three washes with PBS-Tween 20 (0.05% v/v), various concentrations of purified rAFnBPA constructs in PBS were added and incubated at room temperature for 1 h. Wells were washed with PBS-Tween 20 and bound protein detected by incubation with a 1:2000 dilution of polyclonal anti-rAFnBPA$_{37–544}$ antibodies in 5% skimmed milk in TS-Tween 20 for 1 h at room temperature. After three washes with PBS-Tween 20, goat anti-rabbit horse radish peroxidase-conjugated antibodies (1:2000) in 5% skimmed milk in TS-Tween 20 were added and bound peroxidase detected by the addition of 100 μl of 3,3′,5,5′-tetramethylbenzidine (TMB) (0.1 mg ml^{-1}) (Sigma) prepared in 0.05 M phosphate-citrate buffer containing 0.006% (v/v) hydrogen peroxide. Plates were then incubated at room temperature for 10 min. The reaction was stopped by the addition of 50 μl of 2 M H_2SO_4 to each well. Plates were read at 450 nm using an ELISA plate reader (Multiskan EX, Labsystems). Half maximum binding values reported for all ELISA-type binding assays were predicted by the Prism GraphPad software. The statistical significance of the differences between the affinities of proteins was determined by the Student *t*-test, using the online GraphPad software. Differences were considered significant if *P*-values were less than 0.05.

Generation of 3D-model for FnBPA$_{194–544}$

A theoretical model of the structure of region A of FnBPA was obtained by submitting the amino acid sequence for this segment of the protein to the Phyre service of the 3D-PSSM

website (http://www.sbg.bio.ic.ac.uk/phyre/) which models the structure of this sequence based on the crystal structure of the equivalent domains of the *S. aureus* clumping factor ClfA. The accuracy of the structural prediction was estimated by the Phyre programme to be very high (e 1.2^{-33}). A fibrinogen ligand (C-terminal eight amino acid peptide of the γ chain of fibrinogen, G_{404}AKQAGDV$_{411}$) was docked into the predicted structure using Autodock and the most energetically favourable docking solution for the peptide binding into the hydrophobic trench was chosen. All structures were viewed using the UCSF Chimera viewing software. The Autodock programme (http://autodock.scripps.edu/) is designed to predict how small molecules such as drug candidates or peptide ligands bind to a given receptor. This programme has been widely used, is fast and provides high quality predictions. A study on the reliability of the results obtained with Autodock concluded that the programme is successful in predicting protein-ligand complexes (Hetenyi and van der Spoel, 2002). A 3D image of FnBPA docked with the fibrinogen peptide ligand is available as supplementary information.

Construction of rAFnBPA$_{37–511}$ site-directed substituted proteins

The pQE30 expression plasmid containing the DNA sequence encoding rAFnBPA$_{37–511}$ was subjected to site-directed mutagenesis by the Quikchange method (Stratagene). Primers (sequences available on request) containing required base changes were used to amplify the rAFnBPA-containing plasmid. Resulting PCR products were digested with DpnI to remove contaminating wild-type plasmid and then transformed into *E. coli* XL-1 Blue. Transformants were screened by sequencing and recombinant proteins purified from *E. coli* strains as described above. The *L. lactis* pNZ8037*fnbA* plasmid was subjected to site-directed mutagenesis to yield strain NZ9800 expressing full length FnBPA N304A/F306A which was used in bacterial adhesion assays.

Circular dichroism (CD) spectroscopy

rAFnBPA$_{37–511}$ wild type, N304A, F306A and N304A/F306A protein samples were dialysed into 1 mM Tris-HCl pH 7.4. Far UV CD data were collected using a Jasco J-810 spectropolarimeter. Five scans were averaged for each spectrum and the contribution from buffer subtracted in each case. CD spectra were plotted over the 200–300 nm wavelength range and shown to be similar to that of wild-type protein.

Bacterial adhesion to immobilized elastin peptides

Bacterial adhesion to immobilized elastin peptides was performed as previously described (Roche *et al.*, 2004). Briefly, microtitre plate wells (Povair) were coated with 10 μg ml^{-1} of aortic elastin peptides which were then air dried under UV light (366 nM) at room temperature for 18 h. Wells were blocked for 2 h at 37°C with 200 μl of 5% skimmed milk in TS buffer. *L. lactis* cultures expressing FnBPA were washed with PBS and resuspended to an OD = 1 (1×10^8 colony forming units ml^{-1}). Bacterial cell adherence was measured using a fluorescent nucleic acid stain SYTO-13 (Molecular Probes). Bacterial cells were incubated with SYTO-13 (2.5 μM) at room temperature for 15 min in the dark. Elastin-coated wells were washed three times with PBS and 100 μl of stained cells was added to the plate and incubated in the dark for 90 min. Wells were washed three times with PBS and adherent bacteria were measured using an LS-50B spectrophotometer (Perkin-Elmer) with excitation at 488 nm and emission at 509 nm.

Bacterial adhesion to immobilized fibrinogen and fibronectin

Bacterial adhesion to immobilized fibrinogen and fibronectin was performed as described previously (Hartford *et al.*, 2001). Briefly, microtitre plate wells were coated with 10 μg ml^{-1} of human fibrinogen or fibronectin and incubated at 4°C for 18 h. Wells were blocked for 2 h at 37°C with 200 μl of 5% skimmed milk in TS buffer. *L. lactis* cultures expressing FnBPA were washed with PBS and resuspended to an OD = 1 (1×10^8 colony forming units ml^{-1}) and 100 μl of cells were incubated on fibrinogen or fibronectin coated wells for 90 min at room temperature. Adherent bacteria were measured by staining with 5% (w/v) crystal violet for 1 min. Wells were washed with PBS and cell-associated stain was solubilized with 5% (v/v) acetic acid and the A_{570} was determined in an ELISA plate reader.

Inhibition of L. lactis *binding to immobilized elastin/fibrinogen using fibrinogen gamma chain peptide and monoclonal antibody 7C5*

The C-terminal 17 amino acids of the γ chain of fibrinogen (G_{395}EGQQHHLGGAKQAGDV$_{411}$) was synthesized at the Institute of Biopharmaceutical Sciences, Royal College of Surgeons in Ireland, Dublin. A scrambled version of this peptide (Wann *et al.*, 2000) was a gift of M. Hook, Texas A and M University, Houston, Texas, USA. Equal volumes of fibrinogen γ chain peptides (final concentration ranging from 400 μM to 0 μM) or monoclonal antibodies 7C5 and 1F9 (final concentration ranging from 100 μg ml^{-1} – 0 μg ml^{-1}) were preincubated for 1 h with washed *L. lactis* pNZ*fnbA* cultures induced with nisin (1.6 ng ml^{-1} for elastin, 0.8 ng ml^{-1} for fibrinogen) and resuspended to a final OD of 1 before being added to ligand-coated wells for 90 min. Bacteria adhering to elastin and fibrinogen-coated wells were measured by SYTO-13 staining and crystal violet staining, respectively, as described above.

Western immunoblotting

Recombinant FnBPA region A protein samples were boiled for 5 min in an equal volume of 2 × sample buffer before electrophoresis. SDS-PAGE was performed through a 4.5% stacking and 10% polyacrylamide separating gel (Laemmli, 1970). Proteins were transferred to a polyvinylidene difluoride membrane (Roche) by the wet transfer method. Polyclonal anti-rAFnBPA$_{37–544}$ antibodies (1.6 μg ml^{-1}) and monoclonal

antibodies 7C5 (2.5 μg ml^{-1}), 1E6, 1F9 and 1G8 (1.25 μg ml^{-1}) were used to detect recombinant N23 proteins from *S. aureus* strains P1 and 8325–4. Goat anti-rabbit-HRP and rabbit anti-mouse-HRP antibodies were used to detect bound polyclonal and monoclonal antibodies respectively. Peroxidase-conjugated antibodies were detected with LumiGLO chemiluminescence substrate (New England Biolabs) and exposed to X-Omat autoradiographic film (Kodak).

Acknowledgements

We would like to thank Magnus Hook for the gift of a peptide and for helpful discussions, Fondazione CARIPLO and FAR (Fondo d'Ateneo per la Ricerca) for funding to P.S. and the Health Research Board of Ireland for funding to T.J.F.

References

Cox, B.A., Starcher, B.C., and Urry, D.W. (1974) Communication: coacervation of tropoelastin results in fiber formation. *J Biol Chem* **249:** 997–998.

Deivanayagam, C.C., Wann, E.R., Chen, W., Carson, M., Rajashankar, K.R., Hook, M., and Narayana, S.V. (2002) A novel variant of the immunoglobulin fold in surface adhesins of *Staphylococcus aureus*: crystal structure of the fibrinogen-binding MSCRAMM, clumping factor A. *EMBO J* **21:** 6660–6672.

De Ruyter, P., Kupiers, O.P., and De Vos, W.M. (1996) Controlled gene expression systems for *Lactococcus lactis* with the food grade inducer nisin. *Appl Environ Microbiol* **62:** 3662–3667.

Downer, R., Roche, F., Park, P.W., Mecham, R.P., and Foster, T.J. (2001) The elastin-binding protein of *Staphylococcus aureus* (EbpS) is expressed at the cell surface as an integral membrane protein and not as a cell wall-associated protein. *J Biol Chem* **277:** 243–250.

Fitzgerald, J.R., Loughman, A., Keane, F., Brennan, M., Knobel, M., Higgins, J., *et al.* (2006) Fibronectin-binding proteins of *Staphylococcus aureus* mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIIb/IIIa and IgG binding to the FcgammaRIIa receptor. *Mol Microbiol* **59:** 212–230.

Fowler, V.G., Jr, Miro, J.M., Hoen, B., Cabell, C.H., Abrutyn, E., Rubinstein, E., *et al.* (2005) *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA* **293:** 3012–3021.

Gomez, M.I., O'Seaghdha, M., Magargee, M., Foster, T.J., and Prince, A.S. (2006) *Staphylococcus aureus* protein A activates TNFR1 signaling through conserved IgG binding domains. *J Biol Chem* **281:** 20190–20196.

Hartford, O., Francois, P., Vaudaux, P., and Foster, T.J. (2001) The dipeptide repeat region of the fibrinogen-binding protein (clumping factor) is required for functional expression of the fibrinogen-binding domain on the *Staphylococcus aureus* cell surface. *Mol Microbiol* **25:** 1065–1076.

Hetenyi, C., and van der Spoel, D. (2002) Efficient docking of peptides to proteins without prior knowledge of the binding site. *Protein Sci* **11:** 1729–1737.

Jonsson, K., Signas, C., Muller, H.P., and Lindberg, M. (1991) Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*. The complete nucleotide sequence and characterization of the second gene. *Eur J Biochem* **202:** 1041–1048.

Kuhn, G., Francioli, P., and Blanc, D.S. (2006) Evidence for clonal evolution among highly polymorphic genes in methicillin-resistant *Staphylococcus aureus*. *J Bacteriol* **188:** 169–178.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Loughman, A., Fitzgerald, J.R., Brennan, M.P., Higgins, J., Downer, R., Cox, D., and Foster, T.J. (2005) Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by *Staphylococcus aureus* clumping factor A. *Mol Microbiol* **57:** 804–818.

McDevitt, D., Francois, P., Vaudaux, P., and Foster, T.J. (1995) Identification of the ligand-binding domain of the surface-located fibrinogen receptor (clumping factor) of *Staphylococcus aureus*. *Mol Microbiol* **16:** 895–907.

O'Connell, D.P., Nanavaty, T., McDevitt, D., Gurusiddappa, S., Hook, M., and Foster, T.J. (1998) The fibrinogen-binding MSCRAMM (clumping factor) of *Staphylococcus aureus* has a Ca2+-dependent inhibitory site. *J Biol Chem* **273:** 6821–6829.

O'Seaghdha, M., van Schooten, C.J., Kerrigan, S.W., Emsley, J., Silverman, G.J., Cox, D., *et al.* (2006) *Staphylococcus aureus* protein A binding to von Willebrand factor A1 domain is mediated by conserved IgG binding regions. *Febs J* **273:** 4831–4841.

Owen, P. (1985) *Crossed Immunoelectrophoresis in the Study of Outer Membrane Antigens. In Enterobacterial Surface Antigens: Methods for Molecular Characterisation*. Amsterdam: Elsevier. Science Publishers B.V.

Park, P.W., Rosenbloom, J., Abrams, W.R., and Mecham, R.P. (1996) Molecular cloning and expression of the gene for elastin-binding protein (ebpS) in *Staphylococcus aureus*. *J Biol Chem* **271:** 15803–15809.

Peacock, S.J., Foster, T.J., Cameron, B.J., and Berendt, A.R. (1999) Bacterial fibronectin-binding proteins and endothelial cell surface fibronectin mediate adherence of *Staphylococcus aureus* to resting human endothelial cells. *Microbiology* **145:** 3477–3486.

Pilka, E.S., Werner, J.M., Schwarz-Linek, U., Pickford, A.R., Meenan, N.A., Campbell, I.D., and Potts, J.R. (2006) Structural insight into binding of *Staphylococcus aureus* to human fibronectin. *FEBS Lett* **580:** 273–277.

Ponnuraj, K., Bowden, M.G., Davis, S., Gurusiddappa, S., Moore, D., Choe, D., *et al.* (2003) A 'dock, lock, and latch' structural model for a staphylococcal adhesin binding to fibrinogen. *Cell* **115:** 217–228.

Roche, F.M., Downer, R., Keane, F., Speziale, P., Park, P.W., and Foster, T.J. (2004) The N-terminal A domain of fibronectin-binding proteins A and B promotes adhesion of *Staphylococcus aureus* to elastin. *J Biol Chem* **279:** 38433–38440.

Rosenbloom, J. (1982) Elastin: biosynthesis, structure, degradation and role in disease processes. *Connect Tissue Res* **10:** 73–91.

Sambrook. J., Fritsch, E.F., and Maniatis. T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold

Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Schwarz-Linek, U., Werner, J.M., Pickford, A.R., Gurusiddappa, S., Kim, J.H., Pilka, E.S., *et al.* (2003) Pathogenic bacteria attach to human fibronectin through a tandem beta-zipper. *Nature* **423:** 177–181.

Sherertz, R.J., Carruth, W.A., Hampton, A.A., Byron, M.P., and Solomon, D.D. (1993) Efficacy of antibiotic-coated catheters in preventing subcutaneous *Staphylococcus aureus* infection in rabbits. *J Infect Dis* **167:** 98–106.

Signas, C., Raucci, G., Jonsson, K., Lindgren, P.E., Anantharamaiah, G.M., Hook, M., and Lindberg, M. (1989) Nucleotide sequence of the gene for a fibronectin-binding protein from *Staphylococcus aureus*: use of this peptide sequence in the synthesis of biologically active peptides. *Proc Natl Acad Sci USA* **86:** 699–703.

Sinha, B., Francois, P.P., Nusse, O., Foti, M., Hartford, O.M., Vaudaux, P., *et al.* (1999) Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin alpha5beta1. *Cell Microbiol* **1:** 101–117.

Wann, E.R., Gurusiddappa, S., and Hook, M. (2000) The fibronectin-binding MSCRAMM FnbpA of *Staphylococcus aureus* is a bifunctional protein that also binds to fibrinogen. *J Biol Chem* **275:** 13863–13871.

Williams, R.E.O. (1963) Healthy Carriage of *Staphylococcus aureus*: Its prevalence and importance. *Bacteriol Rev* **27:** 56–71.

Supplementary material

The following supplementary material is available for this article online:

Fig. S1. The predicted 3D structure of rAFnBPA N23 (select, chain '(no ID)'), based on homology modelling with the equivalent domains of ClfA. The fibrinogen γ-chain peptide (G404AKQAGDV411) (select, chain 'het') has been docked into this structure and is found situated in the hydrophobic trench between N2 and N3.

The file can be viewed using Chimera or similar software to show the protein structure in 3D.

This material is available as part of the online article from http://www.blackwell-synergy.com